Identification of Novel Multidrug Resistance-Associated Protein-3 (MRP3) Inhibitors in Rat Hepatocytes in Suspension

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INTRODUCTION

- A significant number of preclinical and clinical studies pointed out that Multidrug Resistance-associated Protein (MRP) mediated efflux transport plays an important role in the systemic and tissue exposure profiles of many drugs, their metabolites and endogenous compounds like bile acids[1]. A problem associated with the MRP subfamily is that the **exact role** of the various **isoforms** in drug disposition is relatively **hard to study**, at least partly due to **lack of potent and selective MRP inhibitors**[2].

PURPOSE

- The purpose of this study was to **identify selective Mrp3 inhibitors** in **rat hepatocytes** in **suspension** using the **oil spin method**[3].

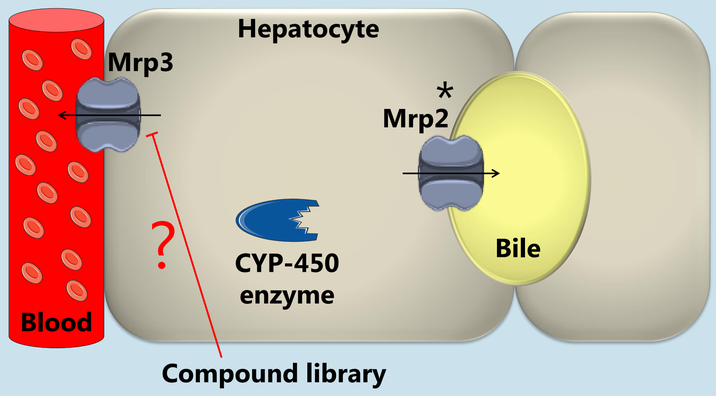
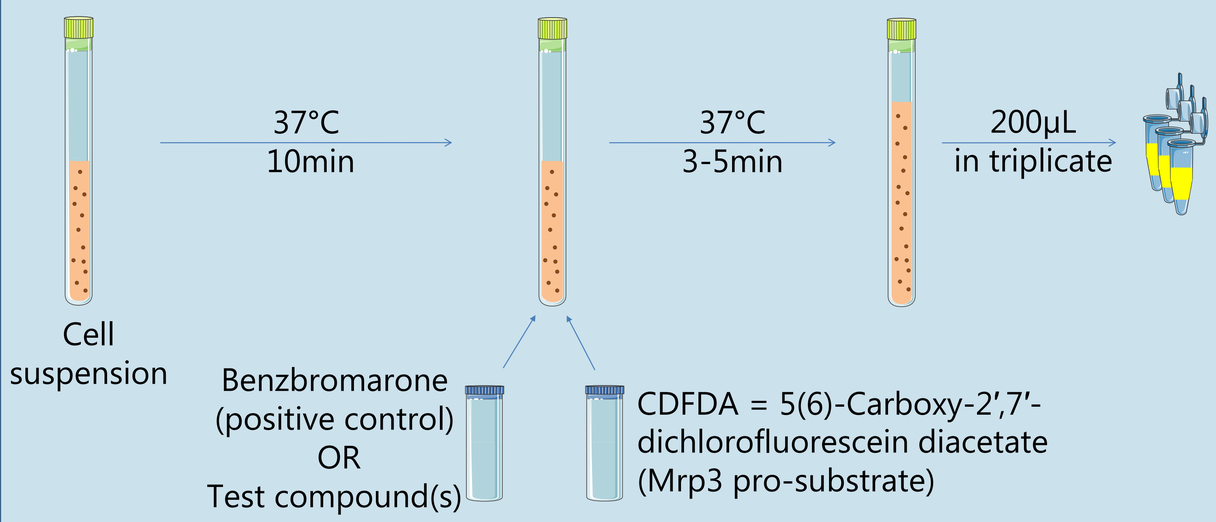


Figure 1: Graphical presentation of the purpose of this study. The aim is to identify selective inhibitors for Mrp3 using a screening approach in rat hepatocytes in suspension.
* In suspension, hepatic Mrp2 activity and expression is very low due to internalisation implying that the present approach will elucidate Mrp3 inhibitors[4].

METHODS

1) Pre-incubation step

2) Incubation step



3) Intracellular CDF quantification (Oil–spin method)

Incubation
CDFDA will react with intracellular esterases and CDF, benzbromarone or possible Mrp3 inhibitor with transporter

Centrifugation
To separate cells from suspension mixture

Quantification
Tube bottoms were cut, lysed and analysed by Fluorescence spectroscopy

Figure 2: Graphical presentation of the optimized assay used to screen for Mrp3 inhibitors in rat hepatocytes in suspension. The Spectrum Collection (MSDiscovery) was used as compound library. In total 1900 compounds were screened.

RESULTS

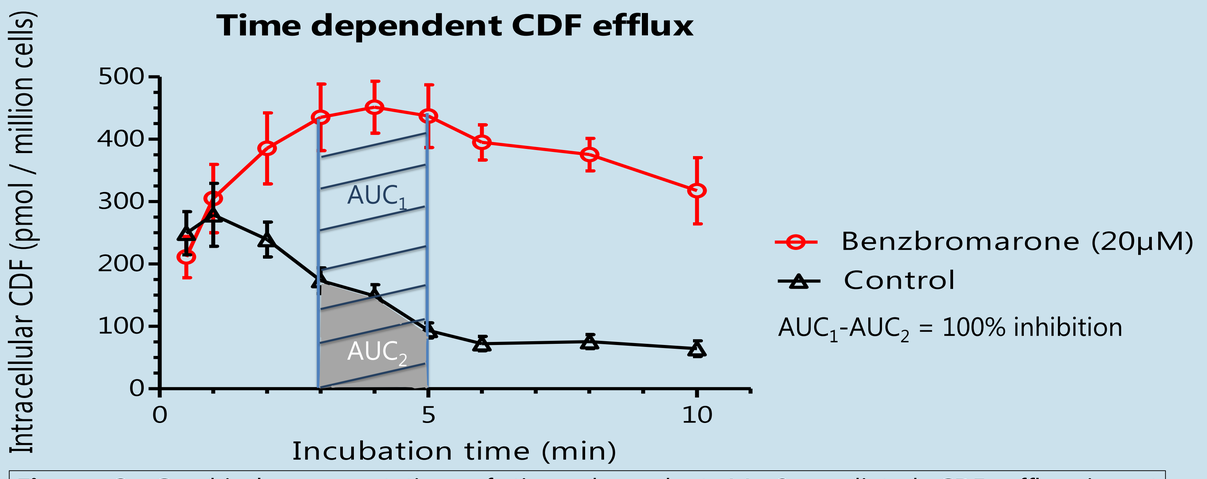


Figure 3: Graphical representation of time dependent Mrp3 mediated CDF efflux in rat hepatocytes in suspension. Based on the results, 3min and 5 min were selected as time points for screening. The area under the curve (AUC) of benzbromarone subtracted by the AUC of the control condition represents 100% inhibition. (n=3)

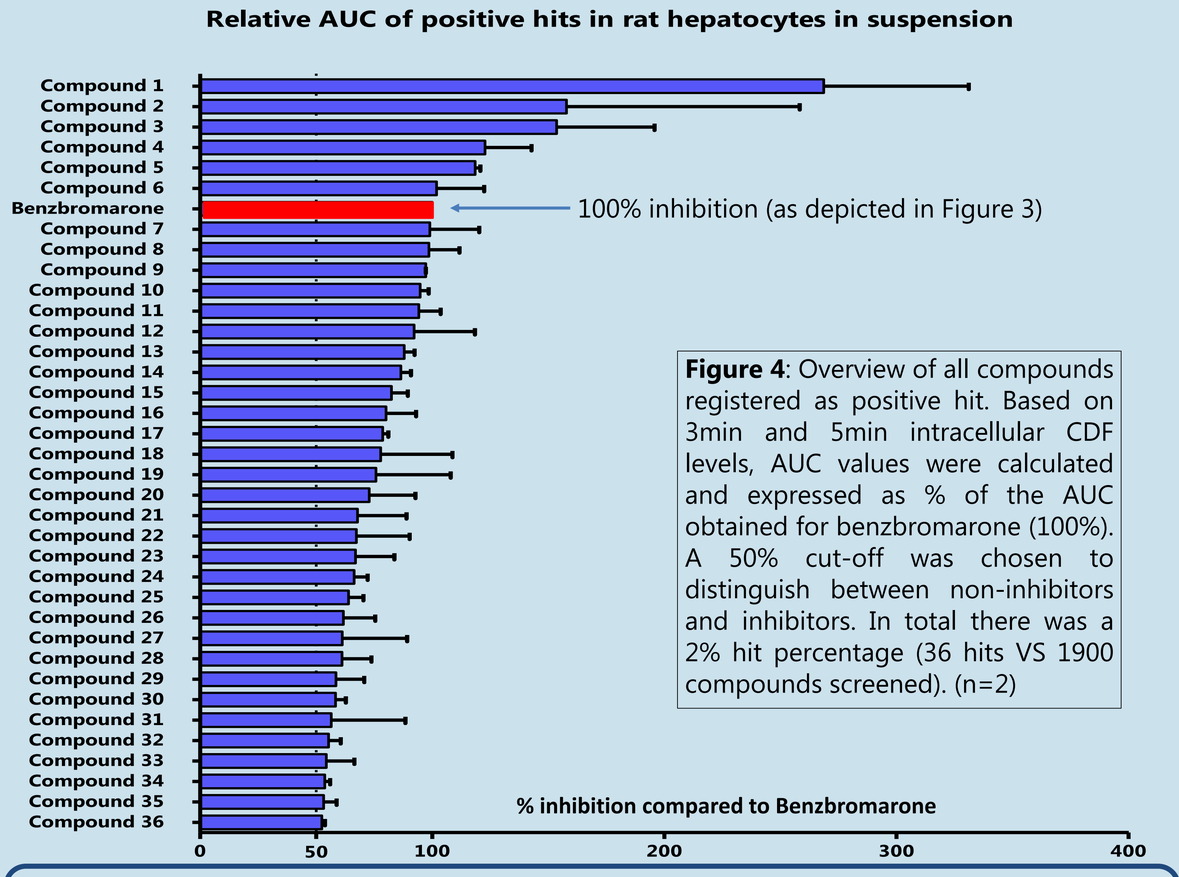


Figure 4: Overview of all compounds registered as positive hit. Based on 3min and 5min intracellular CDF levels, AUC values were calculated and expressed as % of the AUC obtained for benzbromarone (100%). A 50% cut-off was chosen to distinguish between non-inhibitors and inhibitors. In total there was a 2% hit percentage (36 hits VS 1900 compounds screened). (n=2)

CONCLUSION

- Several strong Mrp3 inhibitors were identified using rat hepatocytes in suspension in this **optimized *in vivo* relevant *in vitro* assay.**
- **Human** hepatocytes are currently used which will allow to elucidate **cross-species differences** for MRP3 inhibition.
- **SAR modeling** will enable *in silico* screening larger libraries for more and potent MRP3 inhibitors.

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